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Comparison study between Pilocarpine and Tropicamide drops on corneal topography and their effect on IL-6 and TNF-α levels in tear

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ABSTRACT

Corneal stability is essential for contact lenses and refractive surgery. It seems that paralyzing eye drops or expansion of the ciliary muscle affect the radius of curvature and the strength of the cornea, and this effect is to increase the strength of the cornea during muscle spasm and decrease it in the relaxed state of the muscle. On the other hand, different factors (such as contact lens wear, ocular surface disorders, trauma, dry eye, and immunosuppression) could alter the immune defense mechanisms of the outer eye and permit microorganisms to invade the cornea. Therefore, the present study compared Pilocarpine and tropicamide drop on corneal topography and their effect on IL-6 and TNF-α levels in tear. This prospective study was performed on sixty normal and healthy eyes of sixty volunteers with a mean age of 38.19 years and without any ocular pathology. Volunteers were divided into two groups of thirty. In the first group, corneal topography of both eyes was measured before and 30 minutes after instillation of topical tropicamide 1% in only one eye. The other eye was the control eye, and no drop was given. The same routine was performed in the second group, except that subject received one drop of Pilocarpine 2% in one eye. Statistical comparison between groups for the central corneal power, corneal radius, and corneal astigmatism was performed using paired t-test. IL-6 and TNF-α levels in tear were analyzed using two Luminex commercial assays with Bio-Plex 200TM System (Bio-Rad, Hercules, California, USA). In group 1, no significant changes were found in corneal radius, power, and astigmatism. However, in group 2 subjects who received pilocarpine eye drops, the mean corneal radius value decreased significantly by 0.05 mm. The mean corneal power increased by +0.32 D. There was no significant difference change in corneal astigmatism in both groups. Evaluation of IL-6 levels in tears showed a significant difference between the control and treatment groups (P = 0.041). But no significant difference was observed between the Pilocarpine and the Tropicamide groups (P = 0.761). Evaluation of TNF-α level in tears also showed no significant difference between these groups (P = 0.088). Pilocarpine induced ciliary muscle contraction, which may cause pressure on the corneal limbus and scleral spur, resulting in changes in corneal curvature. But tropicamide eye drop did not affect corneal radius and other corneal parameters, and corneal topography can be carried out after the installation of tropicamide eye drop.

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Introduction

The anterior surface of the cornea has often undergone various studies due to its enormous contribution to the overall refractive power of the eye. Corneal stability is vital for contact lenses and refractive surgery (1). The researchers report that corneal topography provides more information about the corneal surface curvature than other available techniques. Using this advanced computer topography system, thousands of measurements are taken and analyzed in a short time (2). The color map of this information helps evaluate and examine the changes in the anterior and posterior surfaces of the cornea and its thickness following refractive surgeries and orthokeratology and corneal diseases (3).

Corneal topography changes have been reported after LASIK, radial keratotomy, and corneal transplantation. Also, continuous use of contact lenses, hard lenses, causes corneal topographic changes that are often reversible after removal of the lens (4). Researchers have studied corneal topographic changes following the effects of pilocarpine and tropicamide eye drops (5). Previous studies have not shown significant changes in the cornea using these drugs (6). The changes in corneal curvature obtained in the relevant research are also thought to be due to errors in devices or patients and observers. Still, new studies have shown that some of these drugs can affect the center and around the cornea (7).

Pilocarpine is a parasympathetic mimetic that directly stimulates cholinergic receptors. Pilocarpine causes the iris sphincter muscle to contract, causing the pupil to shrink (8). Contraction of the ciliary muscle increases compliance and decreases intraocular pressure. Pilocarpine is used to treat open-angle glaucoma and closed-angle glaucoma before surgery and to induce pupil narrowing after surgery or ophthalmoscopy (9). Tropicamide is an anticholinergic

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drug that inhibits the iris and ciliary body muscle pupil by inhibiting muscarinic receptors in the iris and ciliary muscle, respectively, and causes ciliary muscle paralysis (10). Tropicamide is one of the most widely used drugs used to dilate the eye's pupil and paralyze the ciliary body muscle in ophthalmic diagnostic procedures, including measuring refractive error and examining the end of the eye well mydriasis required before and after surgery (11).

From this point of view, evaluating the effect of this drug on corneal topography (main strength and curvature) is of great importance in the planning and calculation of refractive surgery (4). The mechanism of these changes after dropping tropicamide in the eye can be that this drop causes the ciliary muscle attached to the back of the scleral spur to expand (12). By expanding this muscle, the tension on the scleral spur also decreases and reduces the cornea's curvature. Conversely, when the pilocarpine drop is injected into the eye, the ciliary muscle also contracts, and the pressure from this contraction can increase the cornea's curvature (13). Previous studies have examined changes in corneal curvature during adaptation by pupil contraction (meiosis) with pilocarpine drops. These changes have also been investigated by placing a close stimulus in the topographic device and keratometer center. In both studies, the results showed that the cornea's curvature thinned, and its strength increased during adaptation (14, 15). The average increase in corneal power in different studies varies from 0.13 diopters to 0.72 diopters (14).

Some research has shown the importance of determining the epidemiological profile and performing microbial identification to describe the predisposing factors to develop microbial keratitis (MK) (16). Different factors (such as contact lens wear, ocular surface disorders, trauma, dry eye, and immunosuppression) could alter the immune defense mechanisms of the outer eye and permit microorganisms to invade the cornea (17). Microorganisms change the protective physical barrier at the ocular surface, invading the cornea and initiating an immune/inflammatory response. Several animal models have studied the immunological mechanisms of ocular damage in MK (18, 19). Studies on human epithelial-derived cells and gram-negative bacteria have suggested that IL-6, IL-8, and tumor necrosis factor (TNF)-α in tear are cytokines involved in immune-induced corneal damage (20, 21).

Changes in the strength and curvature of the cornea have also been studied in various studies using pupil dilator and a ciliary muscle paralyzing drops (including tropicamide, phenylephrine, and homatropine) (22). According to their results, phenylephrine drops had the slightest effect, and homatropine (due to its cycloplegic effect) had the most significant effect on corneal curvature and strength (22, 23). Homatropine flattens the radius of curvature of the cornea and reduces its power to about 0.14 diopters (12). Considering the impact of the drug and the results obtained in previous studies, it seems that paralyzing eye drops or expansion of the ciliary muscle affect the radius of curvature and strength of the cornea, and this effect is to increase the strength of the cornea during muscle spasm and decrease its muscle relaxation (22). Therefore, the present study was performed to compare pilocarpine and tropicamide drops on corneal topography and their effect on IL-6 and TNF-α levels in tear.

Materials and Methods

Studied patients

In this study, 60 volunteer patients referred to the ophthalmology clinic of the ophthalmology hospital were included in the study after performing initial examinations and having 20/20 corrected binocular vision. These individuals did not have any specific disease and were randomly selected from the patients referred to the hospital optometry clinic. First, we examined the number of refractive errors and corrected vision with a slit lamp. Individuals who had improved vision by less than 20/20, had a specific disease of the cornea, used eye lens, and had high eye pressure on slit-lamp examination were not included in the study. Also, people with systemic diseases or dry eye and those with a history of eye surgery were excluded from the study.

Experimental evaluations

Sixty eligible volunteers were included in the study and divided into two groups of 30 people. In the first group, first, the corneal topography (Humphrey® topography device ATLAS Model 995) was taken, and then a 1% tropicamide drop (Keeler, USA) was poured into one eye of these people, and no drug was used in the other eye as a control. About 30 minutes after the drop, corneal topography was performed again in both eyes. In the second group, the same was done, but pilocarpine 2% drops (Alcon Canada) were used in one eye, and no medicine was poured into the other eye. After 30 minutes, corneal topography was taken again from both eyes. The same operator fixed all topographies, and the suspicious topographies were repeated. At the end of the samples, the parameters in the corneal topographies of individuals, including the radius of anterior curvature and central corneal strength, and corneal astigmatism before and after dropping drops in the case and control eyes, were compared and statistically analyzed in both groups.

Evaluating IL-6 and TNF-α levels in tear

Tear collection was performed before any other tests. To collect tear samples, 200µl of normal saline (NS) was instilled into the inferior fornix (without topical anesthetics). More than 100µl of tear fluid and NS was collected with a micropipette at the lateral canthus. The tear samples were collected as soon as possible to reduce the stimulation of the ocular surface. The fluid was placed into a 200-lL Eppendorf tube and kept on dry ice during an examination. Then, the samples were stored at -80°C prior to further analyses to avoid repeated freezing and thawing.

Cytokines were analyzed using two Luminex commercial assays with Bio-Plex 200TM System (Bio-Rad, Hercules, California, USA). The concentrations of IL-6 and TNF-α were determined with an eight-plex assay (Magnetic Luminex Performance Assay, R&D Systems, Minneapolis, MN, USA). A total of 50µl tear sample was required for each assay following the manufacturer’s protocols. Data were recorded and analyzed with the Bio-plex Data Pro (Bio-Rad, Hercules, California, USA).

Statistical analysis

Statistics were analyzed using the SPSS for Mac 25.0 (SPSS Inc., Chicago, IL, USA). Data were presented as means ± SD. The data of inflammatory molecules were

logarithm-transformed for normal distribution. Inflammatory molecule concentrations in the two study groups were compared by the nonparametric Mann–Whitney U test. Correlations between clinical parameters and tear inflammatory molecule levels were analyzed using Spearman correlations. P-values less than 0.05 were considered statistically significant.

Results and discussion

Of the 30 subjects in the first group, 13 were male, and 17 were female with a mean age of 38.49 years (49-19 years) who were studied with tropicamide drops. The average refractive error of these people was -0.73 diopters (-1.75 to +0.50). Changes in the central corneal radius of curvature in this group in the case's eyes (before and after 0.5% tropicamide drops) and the control's eyes are shown in (Table 1A).

The mean difference of corneal radius of curvature in the case eyes (0.08 ± 0.006 mm) was not statistically significant; the mean difference of corneal radius of curvature in the control eyes (0.08 ± 0.006 mm) was not significantly different and was not statistically significant. The mean difference in central corneal strength in the case's eyes (before and after tropicamide drops) was reduced after tropicamide drops (0.47 ± 0.03 diopters). But this small change was not statistically significant. Also, the mean difference in corneal power in control eyes (0.48 ± 0.003 diopters) was not much different and was not statistically significant (Table 1B).

The rate of corneal astigmatism was minimal and not statistically significant in the eyes before and after the drop of tropicamide drops (0.37 ± 0.05 diopters) and in the control eyes (0.34 ± 0.07 diopters) (Table 1C).

In the second group, which studied the changes in curvature and strength of the cornea after pilocarpine drops, out of 30 subjects, 17 were men, and 13 were women, with a mean age of 36.19 years (18 to 49 years). The average refractive error of this group was 1.63 diopters (-2.25 to +1.00). The average difference in the radius of curvature of the cornea in the eyes (before and after dropping pilocarpine drops) was about -0.05 mm, which was a change in reducing the radius of the cornea after dropping pilocarpine drops, which was statistically significant. (P <0.05), and the mean difference in corneal radius of curvature in control eyes (-0.01) was not statistically significant (Table 1D).

The mean difference in central corneal strength in the eyes before and after pilocarpine drops was 0.56 to increase corneal strength after pilocarpine drops, which was

Table 1. Changes in the radius of curvature, central strength, and corneal astigmatism in the subjects.

A				
	Mean radius of cornea curvature (mm)		Mean difference in radius of cornea curvature (mm) (X±SD)	P-value
	Before (X±SD)	After (X±SD)		
Case Group (n=30)	7.60 ± 0.28	7.61 ± 0.28	0.08 ± 0.006	0.341
Control Group (n=30)	7.58 ± 0.29	7.58 ± 0.28	0.08 ± 0.006	0.402
B				
	Mean central corneal strength		Mean difference in central corneal strength (X±SD)	P-value
	Before (X±SD)	After(X±SD)		
Case Group (n=30)	44.41 ± 1.66	44.37 ± 1.65	0.47 ± 0.03	0.281
Control Group (n=30)	44.55 ± 1.71	44.54 ± 1.76	0.48 ± 0.003	0.092
C				
	Mean corneal astigmatism		Mean difference of corneal astigmatism (X±SD)	P-value
	Before (X±SD)	After(X±SD)		
Case Group (n=30)	0.86 ± 0.59	0.80 ± 0.54	0.37 ± 0.05	0.192
Control Group (n=30)	0.92 ± 0.64	0.85 ± 0.58	0.34 ± 0.07	0.321
D				
	Mean radius of cornea curvature (mm)		Mean difference in radius of cornea curvature (mm) (X±SD)	P-value
	Before (X±SD)	After(X±SD)		
Case Group (n=30)	7.67 ± 0.29	7.62 ± 0.26	0.10 ± 0.05	0.014
Control Group (n=30)	7.66 ± 0.26	7.65 ± 0.28	0.09 ± 0.01	0.341
E				
	Mean corneal astigmatism		Mean difference of corneal astigmatism (X±SD)	P-value
	Before (X±SD)	After(X±SD)		
Case Group (n=30)	44.04 ± 1.69	44.37 ± 1.46	0.56 ± 0.32	0.003
Control Group (n=30)	44.13 ± 1.55	44.08 ± 1.65	0.37 ± 0.05	0.083
F				
	Mean corneal astigmatism		Mean difference of corneal astigmatism (X±SD)	P-value
	Before (X±SD)	After(X±SD)		
Case Group (n=30)	1.13 ± 0.50	1.09 ± 0.63	0.57 ± 0.03	0.875
Control Group (n=30)	1.02 ± 0.66	1.09 ± 0.73	0.35 ± 0.07	0.908

statistically significant ($p < 0.05$) (Figure 1). But the mean difference in corneal strength in control eyes (0.37) was not statistically significant (Table 1E). The rate of corneal astigmatism did not change significantly in the case eyes, before and after pilocarpine drops (0.57), and in the control eyes (0.33). It was not statistically significant (Table 1F).

Evaluation of IL-6 levels in tears showed a significant difference between the control group and the treatment group ($P = 0.041$). But no significant difference was observed between the Pilocarpine and the Tropicamide groups ($P = 0.761$). Evaluation of TNF-α level in tears also showed no significant difference between these groups ($P = 0.088$) (Figure 2).

Since corneal topography is a powerful tool for assessing small changes in corneal strength and curvature, there are conflicting reports about the effects of ophthalmic drugs on the cornea (4). This study investigated the effect of ciliary muscle contraction and pupil meiosis similar to what occurs in ocular adaptation due to pilocarpine drops. Also, these changes during ciliary muscle expansion and pupil mydriasis, such as what happens in adaptive expansion, have been studied by tropicamide drops with a larger sample volume and a control group than in previous studies.

Our findings in this study showed that the administration of tropicamide drops did not significantly change the strength and curvature of the cornea, which was consistent with the results of research by Sun *et al*. (24) during their study, they did not find significant changes in the strength and curvature of the cornea. They considered the low observed differences due to device error or measurement error. In other studies, however, with more potent cycloplegic drugs, the radius of curvature of the cornea increased, and its main strength decreased slightly but significantly. In Prasannakumary *et al*. (25) study, they instilled the homatropine 2% drop in one eye six times every 10 minutes. Finally, according to the results, this drop and its solid paralyzing effect on the ciliary muscle significantly reduced the tension on the scleral spur and strength in the cornea.

In the present study, this reduction in corneal strength was minimal and was not statistically significant. The reason may be that the used cycloplegic drug was not potent, the amount of its use was tiny (once), and as a result, it had a more negligible effect on ciliary muscle paralysis. However, administration of pilocarpine drops in the eyes of individuals reduced the radius of curvature of the cornea and, in other words, increased its strength, which was statistically significant. These results were confirmed by the research of Saitoh *et al*. (26) (Pilocarpine 2%) and Yasuda *et al*. (27) (Pilocarpine 4%). However, Saitoh's research lacked a suitable control group in this field, and in the study of Yasuda *et al*. (27), a small sample size was used. Kinney *et al*. (28) also reported an increase in corneal strength after pilocarpine drops but attributed the change to decreased intraocular pressure. In another study, the effect of reducing ocular pressure on anterior corneal curvature was assessed by timolol drops (29). Their results showed that lowering eye pressure did not affect anterior corneal curvature. In addition, the time of onset of the pupil tightening effect by pilocarpine drops is 10-30 minutes after consumption. The time to reach the maximum effect (reduction of intraocular pressure) is 75 minutes. In this study, re-topography was performed about 30 minutes after dropping. Therefore, reducing eye pressure cannot

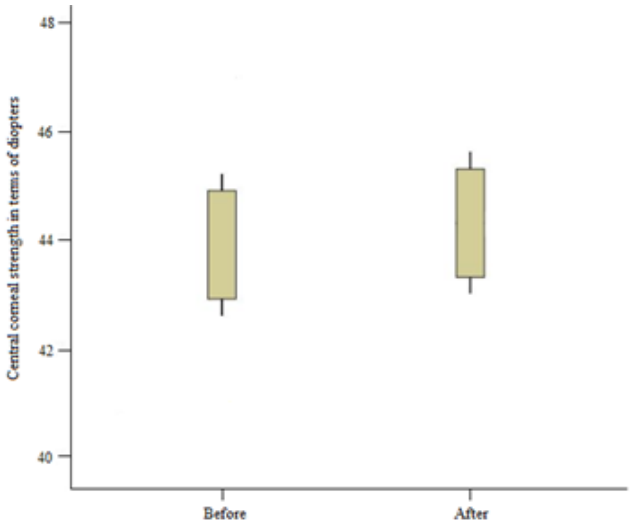

Figure 1. Box diagram of changes in central corneal strength before and after pilocarpine drops.

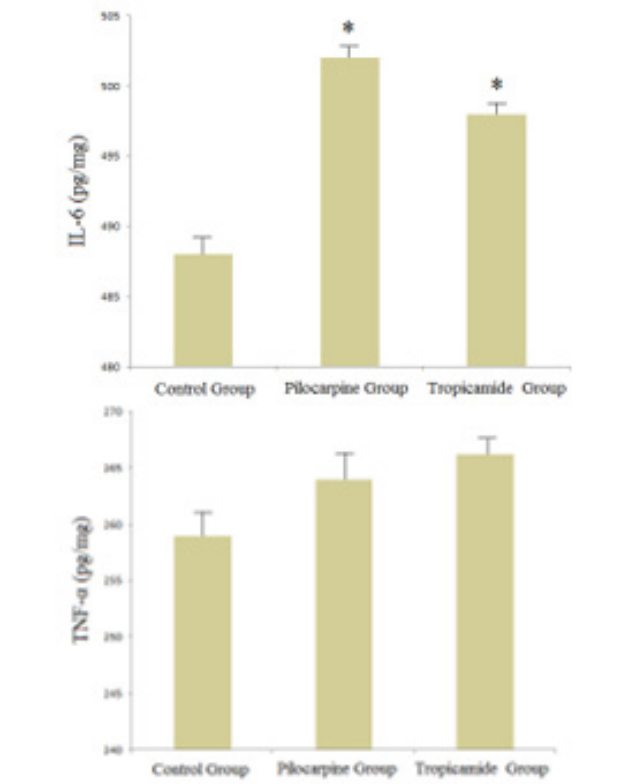

Figure 2. The level of IL-6 and TNF-α in tear among the control group, Pilocarpine group, and Tropicamide group; * means $P < 0.05$ compared to the control group.

be responsible for changing the cornea's curvature after dropping the drop. A similar study on chick eyes showed that the cause of adaptation was ciliary muscle contraction and not a change in eye pressure (30).

In other studies, changes in corneal strength were examined by placing a target near the center of the topographic device or by embedding a mirror in the topographic device or keratometer (31). According to the results, corneal strength increased significantly during adaptation. Buehren *et al*. (32) attributed these changes to the cyclomatic movement during transformation. In this study, the torsional motion did not use pilocarpine drops, and the interfering factor did not affect the results. Also, in this

study, no significant change in corneal astigmatism was observed after dropping pilocarpine and tropicamide drops in the eyes of individuals, which is consistent with the results of previous research on changes in corneal astigmatism during adaptation and adaptive relaxation.

Although it was better to analyze the changes in astigmatism, the number of changes in its axis after dropping the drops was considered, and the method of alpine analysis was used, but this was not possible in this study. However, the rate of change in the axis of astigmatism after dropping drops in patients' eyes was not more than 5 degrees on average.

According to the results of this study, corneal strength increased during ciliary muscle contraction and pupil meiosis due to pilocarpine drops. This contraction of the ciliary muscle causes the scleral spur and the trabecular and peripheral network of the cornea to stretch and ultimately increases the cornea's curvature (33). The findings support the hypothesis that changing the cornea's curvature is also effective in increasing ocular strength during adaptation. Furthermore, the results showed that the expansion of this muscle and pupil mydriasis using tropicamide drops had little effect on corneal curvature. Since this drop is used to perform refractive errors before refractive surgery, it can be ensured that the corneal topography for surgery can be done after using this drop.

In addition, the results of this study can explain the phenomenon of false matching in pseudophakic individuals. According to studies, the eyes of pseudophakic people can have a small amount of adaptation (+0.5 diopters) with subjective measurements. This study estimated the extent of transformation due to pupil meiosis and increased focus depth.

With the results obtained in this study, it can be said that the contraction of the ciliary muscle while trying to adapt can cause an increase in corneal curvature and a slight increase in corneal strength, which can cause false adaptation in pseudo-phakic eyes. Still, this theory needs further investigation and evidence.

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Authors' contribution
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The authors declare that they have no conflict of interest.

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Availability of data and materials
The data used to support the findings of this study are available from the corresponding author upon request.

Statements and Declarations
The author declares that no conflict of interest is associated with this study.

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